Conferences and Reviews

New Insights Into Mechanisms of Immune Glomerular Injury

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Although glomerular disease remains the most common cause of end-stage renal disease worldwide, major advances have been made recently in understanding the cellular and molecular mechanisms that mediate these disorders. The nephrotic syndrome in noninflammatory lesions such as minimal change or focal sclerosis and membranous nephropathy results from disorders of the glomerular epithelial cell that can be simulated in animal models by antibodies to various epithelial cell membrane epitopes. Clarification of how these antibodies affect epithelial cells to induce a loss of glomerular barrier function should substantially improve understanding of the pathogenesis of minimal change or focal sclerosis. In membranous nephropathy, proteinuria is mediated primarily by the C5b-9 complex through similar mechanisms that also involve glomerular epithelial cells as targets.

Inflammatory glomerular lesions are induced by circulating inflammatory cells or proliferating resident glomerular cells. Understanding of how these cells induce tissue injury has also evolved considerably over the past decade. Neutrophil-induced disease involves leukocyte adhesion molecules in regulating neutrophil localization; proteases, oxidants, and myeloperoxidase in mediating injury; and platelets in augmenting these processes. The activated mesangial cell exhibits altered phenotype and proliferation with the release of oxidants and proteases. Mesangial cell proliferation may be initiated by basic fibroblast growth factor and is maintained by an autocrine mechanism involving platelet-derived growth factor. Transforming growth factor β is important in the subsequent development of sclerosis.

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Major advances in the basic sciences of immunology, cell biology, and molecular biology over the past decade have led to major new insights into the pathogenesis of kidney diseases. In this review I focus on current understanding of immunologic glomerular diseases, emphasizing insights derived from recent studies, particularly as they relate to clinical glomerular diseases. In reviewing this area, I focus primarily on the mechanisms that cause acute rather than chronic progressive disease and particularly on proteinuria as a marker of glomerular injury.* I further divide the mechanisms of glomerular tissue injury into those that are associated with inflammatory changes by light microscopy and those that are noninflammatory in nature. The major pathways of injury as they are currently understood are depicted schematically in Figure 1. More detailed reviews of this subject have been published elsewhere.[1-3]

*See also the editorial by D. H. Lovett, MD, "Immunologic Glomerular Disease—New Prospects for Specific Therapy," on pages 481-482 of this issue.

Noninflammatory Mechanisms of Glomerular Injury

Two major glomerular diseases occur in which glomerular permeability is massively increased in the absence initially of any alterations in glomerular structure. These are minimal change or focal sclerosis and idiopathic membranous nephropathy. The glomerular lesions in both these diseases can be closely simulated in animal models in which the principal site of immune attack is the glomerular epithelial cell.

Glomerular Injury Due to Non-Complement Fixing Antibodies to Glomerular Epithelial Cells

The pathogenesis of minimal change or focal sclerosis is unknown. There is now increasing evidence, however, that the nephrotic syndrome results from glomerular effects of circulating permeability factors probably derived from immunocompetent cells. Thus, hybridomas of immunocompetent T cells from patients with active mini-

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ABBREVIATIONS USED IN TEXT

Ig = immunoglobulin
PDGF = platelet-derived growth factor
SPARC = secreted protein acidic and rich in cysteine

mal change disease secrete a factor that can transfer a similar lesion to rats, whereas supernatants from T-cell hybridomas of patients in remission have no such factor.[4] With the use of a new technique that permits the direct measurement of changes in albumin permeability in isolated normal glomeruli, patients with active focal sclerosis evidenced by the recurrence of disease in renal transplants have been found to usually exhibit a serum factor that can induce an increase in albumin permeability.[5] It is unclear whether these two techniques for measuring nonimmunoglobulin permeability factors are measuring the same substance, and the precise characterization of these factors awaits further study. The ability to detect such factors in a clinical setting may represent a major advance in understanding the pathogenesis and treatment of these disorders.

Although these circulating permeability factors are not immunoglobulins, lesions that closely simulate the structural and functional features of minimal change or focal sclerosis can be induced in animals by various antibodies that have in common the ability to bind to the membrane of glomerular epithelial cells without fixing complement. These antibodies, which include some antiglomerular antibodies,[6,7] anti-Fx1A $F(ab')_2$,[8] and monoclonal antibodies K9/9[9,10] and 5-1-6,[11] all induce a dramatic change in the shape of the epithelial cells, accompanied by areas of detachment of epithelial cells from underlying glomerular basement membrane.

A better understanding of how these antibodies cause glomerular injury will likely also clarify how nonimmunoglobulin permeability factors damage glomeruli as well. These models provide the opportunity to conduct such further studies. Possible mechanisms include stimulation of the release by glomerular epithelial cells of materials that directly damage underlying glomerular basement membrane, such as oxidants[12] or proteases,[13,14] or interference with molecules that regulate cell-matrix

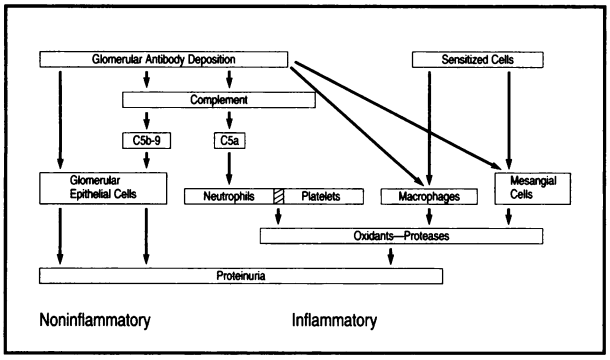

Figure 1.—The schema depicts the established mechanisms of immune glomerular injury that lead to proteinuria (modified from Couser[3]).

interaction leading to cell detachment from the basement membrane,[15,16] or both. Much has been learned recently regarding the molecular mechanisms that regulate the adherence of glomerular epithelial cells to basement membrane, and disorders of these processes may be particularly important in the pathogenesis of noninflammatory types of injury in diseases such as minimal change disease.[16] Detachment is a common underlying feature of many experimental glomerular diseases involving the epithelial cell as a target of injury. Detachment itself may increase protein excretion through glomerular hemodynamic changes, including loss of the hydraulic conductivity barrier and increased water flux that produces enhanced bulk flow of macromolecules, including proteins, through the capillary wall.[17,18]

Glomerular Injury Induced by Complement Fixing Antibodies to Glomerular Epithelial Cells and C5b-9

The prototype of C5b-9-induced glomerular injury in human disease is membranous nephropathy.[19-22] Like minimal change or focal sclerosis, the initial glomerular lesion in membranous nephropathy is also a totally noninflammatory one. In membranous nephropathy, however, there are extensive subepithelial deposits of antibody and complement components including C3 and C5b-9.[22] Although the nature of the deposited antibody in human membranous nephropathy has not yet been established, many other aspects of the immunopathogenesis of the disorder are now understood based on studies of the Heymann nephritis models in rats that closely simulate the human lesion. In Heymann nephritis, subepithelial immune deposits result from antibody binding to antigens on the surface of glomerular epithelial cells, the best characterized of which is GP330 localized in the clathrin-coated pits.[23] Antibody binding to the epithelial cell membrane induces complement activation, probably through the alternative complement pathway.[24] Because the deposits form only on the outer, or subepithelial, surface of the glomerular capillary wall, complement- and immunoglobulin-derived chemotactic and immune adherence proteins are not interactive with circulating cells, probably accounting for the noninflammatory nature of the lesion.[25] Proteinuria, however, is a complement-dependent phenomenon and appears to be mediated primarily by the C5b-9 membrane attack complex of complement (reviewed by Couser and co-workers[19,21]).

Evidence for this derives from studies in intact animals, isolated perfused kidneys, and, recently, isolated glomeruli. In intact animals, selectively depleting C6 with a monospecific antibody abrogates the formation of the C5b-9 complex without other known biologic effects.[26] When rats are given experimental membranous nephropathy by the passive administration of anti-Fx1A antibody (passive Heymann nephritis), C6 depletion has no effect on glomerular antibody deposition but totally abrogates C5b-9 formation in glomeruli and the development of proteinuria.[26] In isolated perfused rat kidneys, the same anti-Fx1A antibody induces proteinuria within 60 min-

utes when all components of the C5b-9 complex are present, but fails to do so in the absence of C6 or C8.[27] Similar results have now been obtained in isolated glomeruli where anti-Fx1A antibody induces a pronounced increase in albumin permeability in the presence of serum that has all components of the C5b-9 complex, but fails to do so in the absence of C6 or C7.[28]

The mechanism by which C5b-9 causes proteinuria is still unclear. Like the permeability factors discussed earlier, the effect of C5b-9 in membranous nephropathy is on the glomerular epithelial cells, where the binding of antibody specific for epithelial cell membrane antigens induces complement activation. Freeze-fracture studies demonstrate the insertion of C5b-9 into the glomerular epithelial cell membrane.[29] In non-nucleated cells, this leads to pore formation and osmotic lysis. In nucleated cells, defense against complement attack is present in the form of complement regulatory proteins such as C8 binding protein and CD59, and sublytic C5b-9 attack may serve instead to activate cells. In glomerular epithelial cells, sublytic C5b-9 attack induces increased intracellular calcium, the activation of phospholipase C, increased levels of inosine phosphorylase 2 and 3, diacylglycerol, and phosphatidic acid and the release of arachidonic acid, prostaglandin $F_{2\alpha}$, and thromboxane.[30-32]

This process of glomerular epithelial cell activation by noncytotoxic C5b-9 attack may lead to the release of mediators that directly damage glomerular basement membrane such as oxidants[33] or proteases.[14,33] Current evidence would support epithelial cell oxidant release as the most likely mechanism. Thus, recent evidence of increased oxidant production by glomerular epithelial cells in experimental membranous nephropathy following C5b-9 attack in vivo has been presented,[35] and the treatment of rats with antioxidants such as dimethyl thiourea substantially reduces proteinuria without reducing antibody deposition or complement activation.[36]

Another possible effect of sublytic C5b-9 is on glomerular epithelial cell attachment to basement membrane. Some studies suggest that proteinuria in membranous nephropathy occurs in areas of detachment.[37,38] Although the cell adherence molecules that regulate glomerular epithelial cell detachment have not been fully identified, epithelial cells appear to express primarily $\alpha_2\beta_1$ and $\alpha_3\beta_1$ integrins,[38,39] and antibody to $\alpha_3\beta_1$ inhibits attachment to laminin, fibronectin, and collagen by 80% to 90% in vitro.[38] Both antibodies to glomerular epithelial cells and C5b-9, perhaps acting through the release of oxidants or proteases, may induce alterations in cell matrix attachment proteins or their ligands that facilitate cell detachment. The apparent increase of antiadhesive proteins such as *s*ecreted *p*rotein *a*cidic and *r*ich in *c*ysteine (SPARC) in C5b-9-mediated epithelial cell injury may also facilitate this process.[40]

Although C5b-9 may participate in inducing the basement membrane thickening and spike formation that characterizes chronic membranous nephropathy, evidence that this is so is less clear. In vitro, C5b-9 produces an increase in type IV collagen,[41] but it has not been shown to have a demonstrable effect on the production of or gene expression for laminin, the principal constituent of the thickened glomerular basement membrane.[42] In vivo increases in laminin and type I collagen gene expression are partially complement-dependent in experimental membranous nephropathy, but type I collagen is not a major constituent of the glomerular scarring in this disease.[42]

Inflammatory Mechanisms of Glomerular Injury

Most types of glomerulonephritis exhibit striking changes by light microscopy, particularly hypercellularity that may reflect infiltration by circulating inflammatory cells such as neutrophils, monocytes, or platelets or the proliferation of resident glomerular cells, particularly mesangial cells. Examples would include postinfectious glomerulonephritis, immunoglobulin (Ig) A nephropathy, rapidly progressive glomerulonephritis, lupus nephritis, and membranoproliferative glomerulonephritis. A plethora of recent studies establishes that both circulating inflammatory cells and resident glomerular cells can mediate glomerular injury acutely by the release of oxidants, proteases, and probably other chemoattractant and glomerular basement membrane-degrading molecules. Ongoing injury is also augmented by the release of various cytokines and growth factors that result in an increased deposition of extracellular matrix, leading to scarring and sclerosis. The following is a brief summary of recent observations in this area.

Neutrophils

In any setting where antibodies induce complement activation or immunoglobulin deposition at sites accessible to circulating inflammatory cells—such as subendothelial or mesangial immune complex deposits—neutrophil infiltration occurs early.[13,43] It has been known since the 1960s that neutrophils cause much of the antibody-induced injury that follows.[44] Recent studies have substantially clarified how neutrophils localize in glomeruli through interaction with a variety of leukocyte adhesion molecules (selectins, integrins, intercellular adhesion molecules, and vascular cell adhesion molecules) that are displayed on cell surfaces.[45,46] Many of these molecules are induced or overexpressed in response to cytokines and other inflammatory mediators. It has also been recently established that neutrophils localized in glomeruli induce injury by undergoing a respiratory burst that results in the release of toxic oxygen metabolites, particularly hydrogen peroxide.[13] Hydrogen peroxide is nephritogenic by virtue of its ability to interact with neutrophil-derived myeloperoxidase.[47-49] Myeloperoxidase is a cationic molecule that localizes in glomeruli by interacting with negatively charged (anionic) sites and interacts with hydrogen peroxide and a halide to form hypohalous acids that directly damage the glomerular basement membrane.[47-49] It has also been shown that neutrophil-derived proteases such as elastase and cathepsin G, in physiologic concentrations, can induce a notable increase in glomerular permeability without accompanying structural dam-

age.[50] Even more recently it has been appreciated that in some settings platelets are not only essential for the occurrence of neutrophil-mediated injury, they apparently augment this process. The nature of this platelet-neutrophil interaction is still under study.

Macrophages

Macrophages are also present in the early cellular infiltrate in several different inflammatory glomerular lesions.[51-53] Unlike neutrophils, they may localize in response to cytokines derived from both cell-mediated and antibody-mediated immune reactions. The presence of macrophages may reflect an initial T-cell infiltrate and be part of a cell-mediated immune reaction that can occur in the absence of antibody deposition.[52] Alternatively, macrophages may be localized through a variety of chemotactic and immune adherence mechanisms to cause tissue injury, like neutrophils, by the release of oxidants and proteases.[54] Macrophages have been shown to be the principal effector cells in some types of chronic immune complex diseases.[55] Unlike neutrophils, macrophages can also contribute to injury through the release of tissue factor that facilitates both intracapillary fibrin deposition and extraglomerular fibrin deposition leading to crescent formation.[56-58] Finally, macrophages are a potent source of transforming growth factor β, a cytokine that is now thought to play a central role in stimulating the production of extracellular matrix by both glomerular and interstitial cells leading to sclerosis and fibrosis.[59-61]

Lymphocytes

The cellular component of the immune response is an important mediator of several autoimmune diseases (reviewed by Waksman[62]). The limited experimental data available on this important mechanism in glomerular disease are well reviewed elsewhere.[51,63] T cells have been clearly shown to mediate hypercellularity independent of antibody deposition in rats[64,65] and chickens[66,67] and appear to participate in the recruitment of macrophages in some models of macrophage-mediated injury.[68,69] A recently described model of glomerulonephritis induced by immunization with myeloperoxidase provides good evidence that cellular immune mechanisms can mediate a glomerular vasculitis similar to that associated with antimyeloperoxidase antineutral cytoplasmic antibodies in humans.[70]

Platelets

Although the role of platelets in glomerular thrombosis has long been appreciated, recent studies have established a role for them in both neutrophil-mediated and mesangial proliferative glomerulonephritis.[71] We noted that the platelet is a prominent component of glomerular injury induced by neutrophil-dependent mechanisms involving oxidant injury and have recently shown that selectively depleting platelets in a neutrophil-mediated model of subendothelial immune complex nephritis essentially abolishes neutrophil-induced proteinuria without altering glomerular localization of antibody, complement, or neutrophils.[72] Studies using platelets labeled with indium 111 have shown that platelet accumulation occurs immediately in this model, within ten minutes of antibody deposition, and is dependent on complement but not on neutrophils.[73] These findings imply that a functional interaction takes place within the glomerulus itself between the neutrophil and the platelet and that this interaction is required for neutrophil-mediated injury to occur. The nature of this interaction has not been defined. The more recently identified role of platelets as mediators of mesangial cell proliferation in models of mesangial proliferative glomerulonephritis is described in more detail in the following section.

Mesangial Cells

Much attention in recent years has focused on the role of the mesangial cell in mediating both immune and nonimmune types of glomerular injury.[74-76] Mesangial cell proliferation is a prominent feature of glomerular diseases including IgA nephropathy, lupus nephritis, some types of steroid-resistant nephrotic syndrome, and other lesions. Because of the relative ease with which mesangial cells can be grown in vitro, a large amount of literature has developed to document the activation of mesangial cells by various inflammatory mediators including C5b-9, certain types of immune complexes, endotoxin, and various other cytokines and growth factors.[75] Additional in vitro studies demonstrate that the activation or proliferation of mesangial cells can lead to the release of a host of proinflammatory materials including oxidants, proteases, prostaglandins, growth factors, and extracellular matrix components.[76]

To initiate studies of the mechanisms that regulate mesangial cell proliferation in vivo, a model of mesangial proliferative glomerulonephritis in rats induced by administering antibody to the Thy 1.1 antigen on the mesangial cell membrane was used. In this antithymocyte serum model, antibody and complement induce an initial mesangiolysis followed by glomerular proliferation that is maximal five days later.[77] Cell proliferation can be accurately quantitated using a stain for the proliferating cell nuclear antigen, a nuclear protein expressed during the late G1 to G2/M phase of the cell cycle.[77] Immunohistochemical colabeling techniques documented that more than 85% of proliferating glomerular cells were mesangial cells as assessed by labeling of cells positive for proliferating cell nuclear antigen with antibody to Thy 1 or to α-smooth muscle actin, a protein expressed de novo by the activated mesangial cell in vivo.[77,78]

A prominent feature of the antithymocyte serum model is an early platelet influx into capillary loops and mesangial areas.[77,79] One of our initial questions was whether the platelet infiltration might somehow mediate the mesangial cell proliferation that followed it. Selective platelet depletion studies showed more than 70% reduction in mesangial cell proliferation in platelet-depleted animals.[77] (Complement depletion also prevented cell proliferation, apparently by markedly reducing the glomerular platelet influx, a previously unrecognized role for the complement system in glomerular injury.[80])

We then hypothesized that the mechanism of this platelet-mediated mesangial cell proliferation might involve the release of growth factors. Based on in vivo studies, platelets are known to produce a number of growth factors that can stimulate mesangial cell proliferation in vitro, including platelet-derived growth factor (PDGF), interleukin-1, epidermal growth factor, insulinlike growth factor type 1, and transforming growth factor β (in low concentrations).[81] We elected to initiate our studies of growth factor effects on mesangial cell proliferation in vivo with PDGF. Platelet-derived growth factor has been well documented by several laboratories to stimulate mesangial cell proliferation in vitro.[82] Using immunohistochemical techniques, we were able to show a substantial increase in mesangial cell production of both PDGF B chain and PDGF receptor β-subunit at three and five days in the antithymocyte serum model.[83] Moreover, when molecular techniques were used, a considerable increase in gene expression for both PDGF A and B chains and PDGF receptor β-subunit was shown in whole glomeruli at three and five days.[83] In situ hybridization studies localized the increase in PDGF B chain messenger RNA to mesangial areas.[84] The time course of detectable increases in both PDGF and PDGF receptor protein and messenger RNA corresponds closely with the course of mesangial cell proliferation in vivo.[83,84] Moreover, maneuvers that blocked mesangial cell proliferation including platelet and complement depletion also blocked these increases in PDGF and its receptor.[83]

In total, our studies provided strong circumstantial evidence in support of the hypothesis that PDGF was indeed an important mediator of mesangial cell proliferation in vivo. Two more studies were needed to confirm this, however. In the first, we attempted to reproduce mesangial cell proliferation by infusing PDGF into the renal artery in vivo. Infusing recombinant PDGF resulted in only modest mesangial cell proliferation in normal rats. If, however, rats were treated with small doses of antithymocyte serum that produced no detectable disease alone, infusing similar doses of PDGF resulted in a much more dramatic mesangial cell proliferation and glomerular hypercellularity.[85] Finally, attempts were made to block mesangial cell proliferation in the antithymocyte serum model by employing a neutralizing antibody to PDGF. To our surprise, we found no effect of PDGF blockade at two days, but a dramatic (60%) reduction in cells positive for proliferating cell nuclear antigen at four days.[86] Our studies thus suggest that PDGF is probably not the principal growth factor that initiates mesangial cell proliferation. Rather, PDGF, probably derived primarily from the mesangial cell itself and not from platelets, contributes to maintaining glomerular hypercellularity in this model by an autocrine mechanism involving mesangial cell stimulation of not only the growth factor but also its receptor.[87,88]

The series of observations summarized above establishes an important role for PDGF in glomerular disease. The demonstration of increased PDGF expression in several proliferative glomerular lesions in humans suggests that these observations are probably relevant in the clinical setting as well.[89] Clearly there are several other growth factors and cytokines that also participate.[75,90] In our laboratory we have begun to look at other factors that may be more important than PDGF in initiating mesangial cell proliferation and found an increase in the release of mesangial cell-derived basic fibroblast growth factor, an intracellular growth factor that can stimulate normal mesangial cells to proliferate in vitro and may be important in initiating this process in vivo.[91] We have also wondered what terminates the autocrine-driven maintenance phase of mesangial cell proliferation that leads to disease resolution. We have studied the role of SPARC in this process. This glycoprotein, which can bind to and neutralize PDGF, is also made by mesangial cells and might be increased as mesangial cell proliferation ensues.[92] Obviously these observations, rather than being answers to the questions posed, are initial steps on what is likely to be a long but, we hope, fruitful journey.

Our studies have also shown that mesangial cell proliferation precedes the increase in gene expression for several normal extracellular matrix components that may contribute to eventual sclerosis, not only in the antithymocyte serum model,[93] but in nonimmunologic progressive glomerular lesions such as the remnant kidney model[94,95] and diabetes mellitus.[96] Not only is matrix synthesis increased, but so, too, are the synthesis and release of a mesangial cell-derived neutral protease with basement membrane-degrading properties.[97] Moreover, a reduction in mesangial cell proliferation induced with antibody to PDGF also affects a reduction in matrix component deposition[86] similar to that seen with reagents that block transforming growth factor β.[58,98]

Conclusion

As understanding of the mechanisms of immune glomerular injury evolves, numerous new therapeutic strategies can now be devised, including agents that block or inhibit complement effects, oxidants, proteases, growth factors, and other cytokines. Appreciation of the role of several natural inhibitors of these mechanisms may also allow therapeutic manipulations that increase the production of regulatory proteins with a consequent therapeutic benefit. Thus, these changes in the basic understanding of the mechanisms of glomerular disease are likely to translate into new and more specific and effective forms of therapy in the next decade.

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